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RESEARCH ARTICLE

Protection of CCl_4-induced hepatic and renal damage by linalool

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ABSTRACT

The aim of the current study is to determine the protective and therapeutic effects of linalool against carbon tetrachloride (CCl_4)-induced hepatotoxicity and nephrotoxicity. Six-week-old male Wistar rats were divided into five groups: Control group (a regular diet); CCl_4 group (1 ml/kg dissolved in olive oil, intraperitoneally at 14th day); pretreatment group (25 mg/kg linalool daily + CCl_4 14thday); post-treatment group (25 mg/kg linalool 2, 6, 24, and 48 h after the injection of CCl_4 at 14th day); and linalool group (25 mg/kg linalool daily, orally). All animals were sacrificed, tissue and blood samples were collected to analysis. Administration of CCl_4 resulted in a marked increase in hepatic (aspartate aminotransferase, alanine transaminase, and alkaline phosphatase) and renal (blood urea nitrogen and creatinine) markers. Also, CCl_4 resulted in pathological damages, a significant increase in the concentration of malondialdehyde , tumor necrosis factor-alpha, and Interleukin 6 , expression of nuclear factor kappa-light-chain-enhancer of activated B cells and a significant decrease in the levels of serum total protein, serum albumin, and antioxidants. However, in pretreatment and post treatment groups, linalool significantly inhibited CCl4- induced hepatic and nephric damages. These results demonstrate that linalool has protective and therapeutic effects in an *in vitro* model of CCL_4-induced hepatic and nephric damage, proposing linalool as a potential therapeutic agent against chemical and drug induced hepatotoxicity and nephrotoxicity.

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Linalool; hepatotoxicity; nephrotoxicity; natural compounds; oxidative stress

Introduction

The liver and kidneys are main organs affected by drug and chemical toxicity (Zhang *et al.* 2007). The liver is the main organ where exogenous and endogenous chemicals are metabolized and finally excreted (Almazroo *et al.* 2017). As a consequence, hepatocytes (liver cells) are exposed to remarkable concentrations of these chemicals and drugs, which can lead to cell death, hepatoxicity, liver dysfunction, and even organ failure (Russmann *et al.* 2009). The kidney has a key role in the regulation of acid-base balance, electrolyte composition, and extracellular fluid volume, and in the excretion of metabolic wastes (Raghavendra and Vidya 2013). Moreover, the kidney synthesizes and releases hormones, and metabolizes vitamin D3 to the active form (Gekle 2017). Therefore, the functional integrity of the mammalian liver and kidneys is vital to total body homeostasis (Burcham 2014). A toxic insult to the liver and kidney could disrupt any or all of these activities and could have profound effects on mammalians (Zhang *et al.* 2012). Therefore, the liver and the kidneys are more commonly involved in overt organ toxicity than most other tissues (Zhang *et al.* 2012). Accidental or deliberate toxicity with drugs can cause life-threatening liver and kidney damages. Moreover, these medicinal risks, liver and kidney injuries accompany exposure to different chemicals in the environment or workplace. Still chemicals such as food contaminants that harm these excretory organs are consumed (Burcham 2014). Therefore, protecting the liver and kidney against these agents is inevitable. Natural compounds found in plants have long been shown to play an important role in inhibiting liver and kidney toxicity (Nale *et al.* 2012).

Herbal medicines have a long history over than 7000 years in the treatment of many diseases. Using natural compounds to treat diseases mainly in the developing countries, is still the pillar of near 75–80% of the treatment in the world (Ekor 2014). Because of better compatibility with the human body, lesser side effects and better cultural acceptability, these compounds are used frequently for primary health care (Ekor 2014). Also, a major increase in their use in the developed countries have seen in the last few years. Nowadays, there is a bipolarized market for the active compounds: those natural ingredients that are requested by patients and those chemically produced by the pharmaceutical companies (Atanasov *et al.* 2015). Many of these agents can have ameliorative effects against drugs and chemicals toxicities (Abdel-Daim *et al.* 2016). One of these natural products is linalool (LIN),

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which found in essential oils of plants such as mint, basil, and coriander. Linalool has several pharmacological effects including antibacterial, antimicrobial, anti-inflammatory, antiviral, anesthetic, and analgesic effects (Sharifi *et al.* 2017). Previous studies showed that linalool, is a competitive antagonist of N-methyl d-aspartate (NMDA) receptors and produces analgesic properties via brain opioids when it inhibits NMDA receptor activity (Batista *et al.* 2008). In addition, the hypolipidemic, antihyperglycemic, and antioxidant properties of linalool, its preventive effect on proteinuria and its therapeutic effect on kidney function were evidenced. It was suggested that some monoterpenes obtained from plants inhibited and decrease tumor growth. Therefore, recent investigations have focused on the chemotherapeutic or chemo-preventive potential of linalool (Kamatou and Viljoen 2008). A recent study has shown that linalool has protective effect against glutamate-induced oxidative stress and excitotoxity (Sabogal-Guáqueta *et al.* 2019). Oner *et al.* (2019) proved that administration linalool with doxorubicin can remove cardiomyopathy symptom induced by this drug. It has been reported that the plasma concentration of linalool reaches about 10 min at the first minor peak, the second major peak reaches in about 40 min, and then the linalool concentration in the plasma decreased rapidly (Shi *et al.* 2016).

Carbon tetrachloride (CCl_4) is widely used as a model for induction of liver and kidney damages in rats to investigate potential therapeutic effect of new agents (Suzuki *et al.* 2015). This chemical is metabolized to active metabolites such as trichloromethyl-free radicals and peroxyl radical by the cytochrome P450 enzymes, which then initiate oxidative damages, cell death, and inflammation (Dai *et al.* 2018a). Whereas, linalool has previously been shown to exhibit anti-inflammatory and antioxidant properties and to the best of our knowledge, there is no literature on the effects of linalool on hepatoxicity and nephrotoxicity. The aim of the present study was to determine the protective and therapeutic effects of linalool, on tetrachloride-induced hepatoxicity and nephrotoxicity in rats using histopathologic, genes analysis and biochemical methods.

Materials and methods

Chemicals and kits

Hydrogen peroxide, methanol, thiobarbituric acid, bovine serum albumin (BSA), Coomassie blue, CCl_4, iron sulfate, ferric chloride, sodium acetate, ketamine, xylazine, and butanol were purchased from Merck Company (Darmstadt, Germany). The Wistar albino rats were purchased from the Baquatollah University of Medical Sciences (Tehran, Iran). The kits for superoxide dismutase (SOD) and glutathione peroxidase (GPx) were purchased from Randox Company (Crumlin, UK). The kits for assaying of aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, bilirubin, serum total protein, and serum albumin levels were purchased from Pars-Azmoon Company (Tehran, Iran). The kits for assaying tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels were purchased from R&D Systems Europe, Ltd. (Abingdon, UK). Linalool (3,7-dimethyl-1, 6-octadien-3-ol) with a purity of about 99% and CAS number 78-70-6 was purchased from Sigma-Aldrich (St Louis, MO). Linalool was freshly prepared before use and dissolved in normal saline 0.9% wt/vol.

Animals

Male Wistar rats weighing 200 ± 20 g was purchased from the Baquatollah University of Medical Sciences (Tehran, Iran) and maintained under a standard dark-light cycle (lights on between 7:00and 7:00am) at room temperature (22 ± 2 °C). These conditions were maintained constantly throughout the experiments. This study was approved by the Ethics Committee at the Ardabil University of Medical Sciences with ethics codes IR.ARUMS.REC.1397.120 and performed strictly in accordance with institutional and international guide for animal care.

Experimental design

Thirty male Wistar rats weighing 200 ± 20 g, aged eight weeks, were selected randomly and given free access to water and food. After two weeks of acclimation, the animals were assigned for experimental procedures. The animals were divided into five subgroups: controls (normal rats $n = 6$) gained free access to food and water; the CCl_4 group ($n = 6$) was given 1 ml/kg of CCl_4, dissolved in olive oil, through intraperitoneally at 14th day; the pretreatment group (25 g mg/kg LIN for 14 days plus single dose of 1 mg/kg CCl_4); the post-treatment group (25 mg/kg LIN 2, 6, 24, and 48 h after the injection of CCl_4 at 14th day), and the LIN group was given normal diet and administered LIN orally (25 mg/kg) for 14 days. The dose of CCl_4 and LIN was determined based on previous studies (Dai *et al.* 2018a, Sabogal-Guáqueta *et al.* 2019). At the end of the experimental procedures, all animals were weighed and then anesthetized using a combination of xylazine (10 mg/kg) and ketamine (10 mg/kg) administered via intraperitoneal (i.p.) injections. Liver and kidney tissues were taken for RNA extraction and gene expression (reverse transcription polymerase chain reaction, RT-PCR) as well as for histopathology and antioxidant capacity.

Serum extraction and chemistry analysis

Blood was collected directly from their heart using heparinized capillary tubes. The blood samples were centrifuged at 3000 rpm for 10 min and the supernatants (serums) were transferred into 0.5 ml vials and were frozen at −80 °C for the subsequent biochemical tests and the analysis of inflammatory factors. Serum creatinine, BUN, bilirubin, albumin, total protein, ALT, AST, and ALP was measured using commercially available kits based on spectrophotometric analysis.

Determination of liver and kidney antioxidant activity

For malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), glutathione

peroxidase (GPx), catalase activity measurements, 200 mg of liver or kidney slices was homogenized in 2 ml of homogenization buffer (50 mM potassium phosphate buffer; PBS, pH 7.4). The homogenized tissues were centrifuged at 10 000 rpm for 2 min. The supernatants were centrifuged again at 12 000 rpm for 20 min to obtain the pure supernatants. The final obtained supernatants were used for the measurement of the antioxidant activity. The activities of SOD, CAT, GPx, and TAC were determined using commercially available kits based on spectrophotometric analysis by StatFax 3000 ELISA reader . Results of SOD, CAT, GPx, and TAC activities were calculated according to the manufacturer's instructions.

Liver and kidney histopathology

At the end of the experimental procedures, livers and kidneys of all experimental animals were removed. The removed tissues were fixed in a buffered neutral formalin solution (10%). Fixed tissues were processed routinely including washing, dehydration, clearing, paraffin embedding, casting, and were sectioned to 4–5 μm thickness for using in hematoxylin and eosin (H&E) staining. To examine the histological alterations 12 images were selected randomly in each sample and the histological index was calculated semi-quantitatively using a scale of 0–3: (0) none, (1) mild, (2) moderate, and (3) severe damage. The investigated damages include degenerated cells, necrotic cells, vacuolation, cell detachment, and lymphocyte infiltration (Peng *et al.* 2018).

Real time quantitative RT-PCR analysis

Total RNA was extracted from liver samples with RNX- Plus (Cat No. EX 6101, SINACLON, Tehran, Iran) according to the kit protocols. Two micrograms of total RNA were reversely transcribed into cDNA by using SMOBIO strand cDNA synthesis kit (lot No #CHRP 051901430-8, Taiwan), according to the manufacturer's instructions. Quantitative RT- PCR was carried out using TB Green Premix Ex TaqTM II (cat#RR820Q, TaKaRa, China) kit on LightCycler® 96 System (Roche Applied Science, Mannheim, Germany). Actb was applied as housekeeping gene for normalization of gene expression data. RT- PCR primer sequences were as follows, Rela: 5′-CCTGTCTGCACCTGTTCCAA-3′ (forward), and 5′-ACTCCTGGGTCTGTGTTGTT-3′ (reverse), Actb: 5′-GGAGAA GATTTGGCACCACACT-3′ (forward), and 5′-CGGTTGGCCTTA GGGTTCAGA-3′ (reverse). The comparative CT method was performed to measure the relative expression levels of Rela in liver samples. All qRT-PCR data were normalized after subtracting the CT values of Rela from that of Actb as an internal control (2-ΔCT method, ΔCT = CTRela – CtActb). Each measurement was performed in triplicate.

Data analysis

The results were analyzed using Graph Pad Prism (version 5, Graph Pad Software Inc., La Jolla, CA). Results are presented as mean ± standard deviation for six independent rats per each group. The statistical significance of the differences between groups was assessed using one-way analysis of variance, *post-hoc* test, and Fisher's least significant difference (LSD) test.

Results

Protective and therapeutic effect of LIN on CCl_4 -induced hepatic and renal histopathological changes

Hepatic changes in control and LIN-administrated showed normal hepatic architecture illustrated by hepatic lobule with

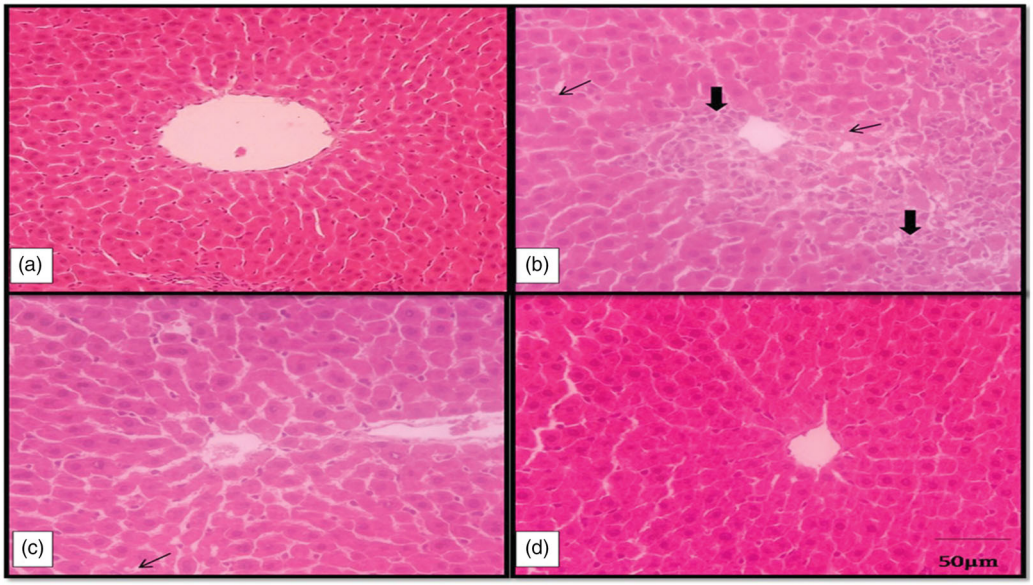

Figure 1. (a) Photomicrograph of the liver of normal control rats shows a normal hepatic architecture represented by hepatic lobule with a thin walled central vein. (b) Photomicrograph of the liver of treated rats with CCl_4 shows restoration of normal hepatic architecture with disappearance of fat droplets from hepatocyte cytoplasm; congestion of the central vein; lymphocytic infiltration; and hydropic degeneration in the hepatocytes, (arrows) and regeneration of hepatic parenchyma. (c) Photomicrograph of the liver of the CCl_4 + Lin group shows disappearance of most of the degenerative changes. (d) Photomicrograph of the liver of Lin group shows normal architecture as that of the control group (H&E, 400×).

a thin walled central vein and normal hepatic cords radiating toward the periphery alternating with hepatic sinusoids. In the CCl_4 group, the livers showed hydropic degeneration in the hepatocytes, lymphocytic infiltration, and congestion of the central vein. The liver of the protective and post treatment group (CCl_4 plus LIN) demonstrated disappearance of the degenerative changes in hepatocytes except few hepatic cells and edema in the central vein showed hydropic degeneration (Figure 1(a–d)). The normal kidney in control and LIN-administered rats showed a normal renal architecture represented by renal tubules and renal corpuscles (Figure 2(a–d)). In the CCl_4-administered group, renal histology showed congestion of the renal blood vessels, degeneration in the cells of the proximal and distal convoluted tubules and vacuolation of the renal tubules (Figure 2). In the protective and posttreatment group, LIN induced recovery and regeneration in renal structure from biohazards of CCl_4 (Figure 2(a–d)). Correspondingly, histological scores were significantly decreased to 2.3 and 1.8 in liver and kidney tissues, respectively compared to CCl_4-treated group (Figure 3).

Protective and therapeutic effect of LIN on CCl_4-induced changes in serum biochemical profiles, hepatic, and renal function tests

The results in Figure 4(a–d);liver) and Figure 4(e–h);kidney) show that oral administration of LIN for 14 days increased the serum levels of ALT, AST, ALP, BUN, creatinine, albumin, bilirubin, and total protein in serum of CCl_4 administered rats compared to control and LIN administered rats. Co-administration of LIN together with CCl_4 normalized such alterations. Moreover, LIN-administered rats did not show increase in serum levels of ALT, AST, ALP, BUN, creatinine, albumin, bilirubin, and total protein. These data indicated that LIN co-administration with CCl_4 restored such alterations to normal levels and LIN has no toxic effects on hepatic, and renal function parameters.

Protective and therapeutic effect of LIN on CCl_4 -induced changes in enzymatic antioxidant defense systems and MDA content of liver and kidney

As shown in the Table 1, in the CCl_4 group, the activity of serum TAC, CAT, SOD, and GPx was significantly ($p < 0.001$) reduced as compared to the control group and LIN group. In the LIN + CCl_4 group, linalool could significantly ($p < 0.001$) ameliorate the activity of these enzymes compared to the CCl_4 group. Also, administration of linalool (25 mg/kg) alone for 14 days caused a significant ($p < 0.001$) increase in the activity of the enzymes as compared to the control group.

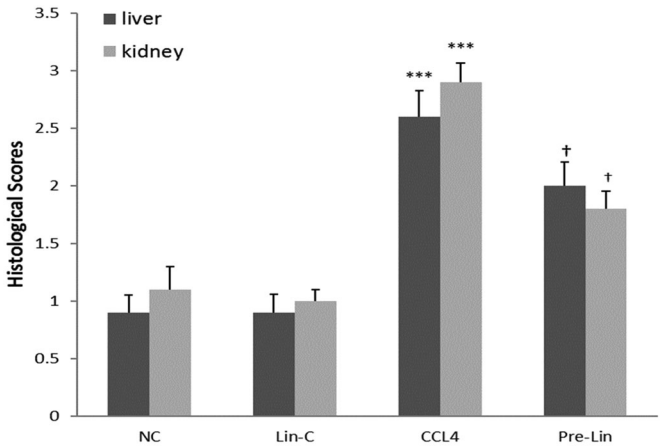

Figure 3. Semi-quantitative analysis of protective and therapeutic effects of Lin against CCl_4 induced the liver and kidney damages. The histological index was calculated semi-quantitatively using a scale of 0–3: (0) none, (1) mild, (2) moderate, and (3) severe damage. The results are expressed as mean ± standard deviation (SD). ***$p < 0.05$ compared with NC group; †$p < 0.05$ compared with CCl_4 group.

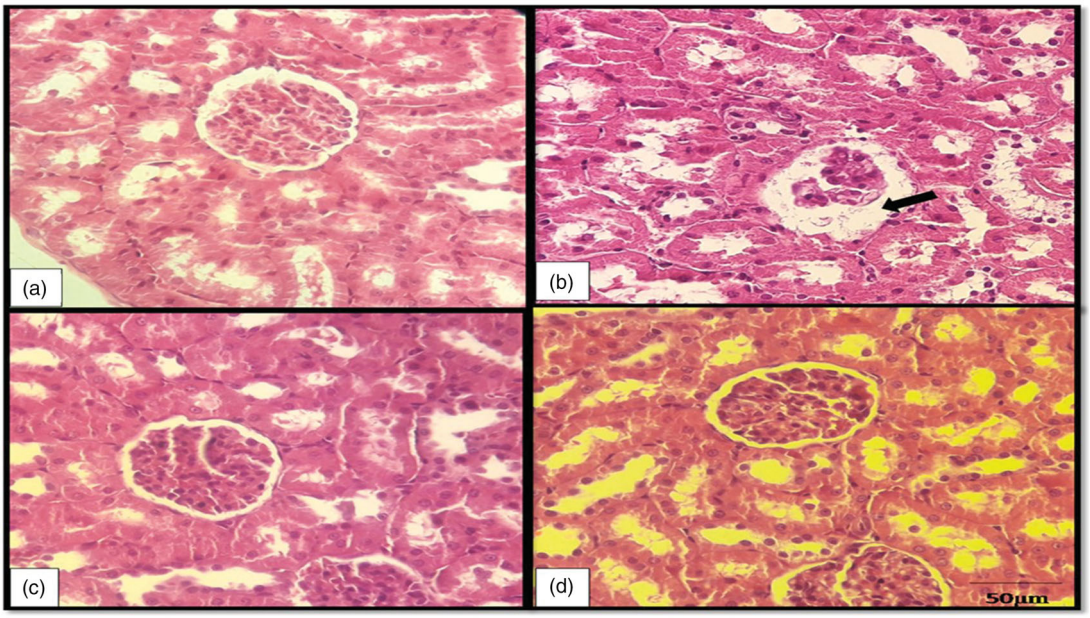

Figure 2. (a and d) Photomicrograph of the kidney of normal control and Lin-administered rats shows normal renal architecture represented by renal tubules and renal corpuscles. (b) Photomicrograph of the kidney of CCL_4-administered rats shows congestion of the renal blood vessels, vacuolation of the renal tubules, and degeneration of the cells of the proximal and distal convoluted tubules. (c) Photomicrograph of the kidney of CCl_4 + Lin group shows recovery of the renal tissue to normal structure (H&E, 400×).

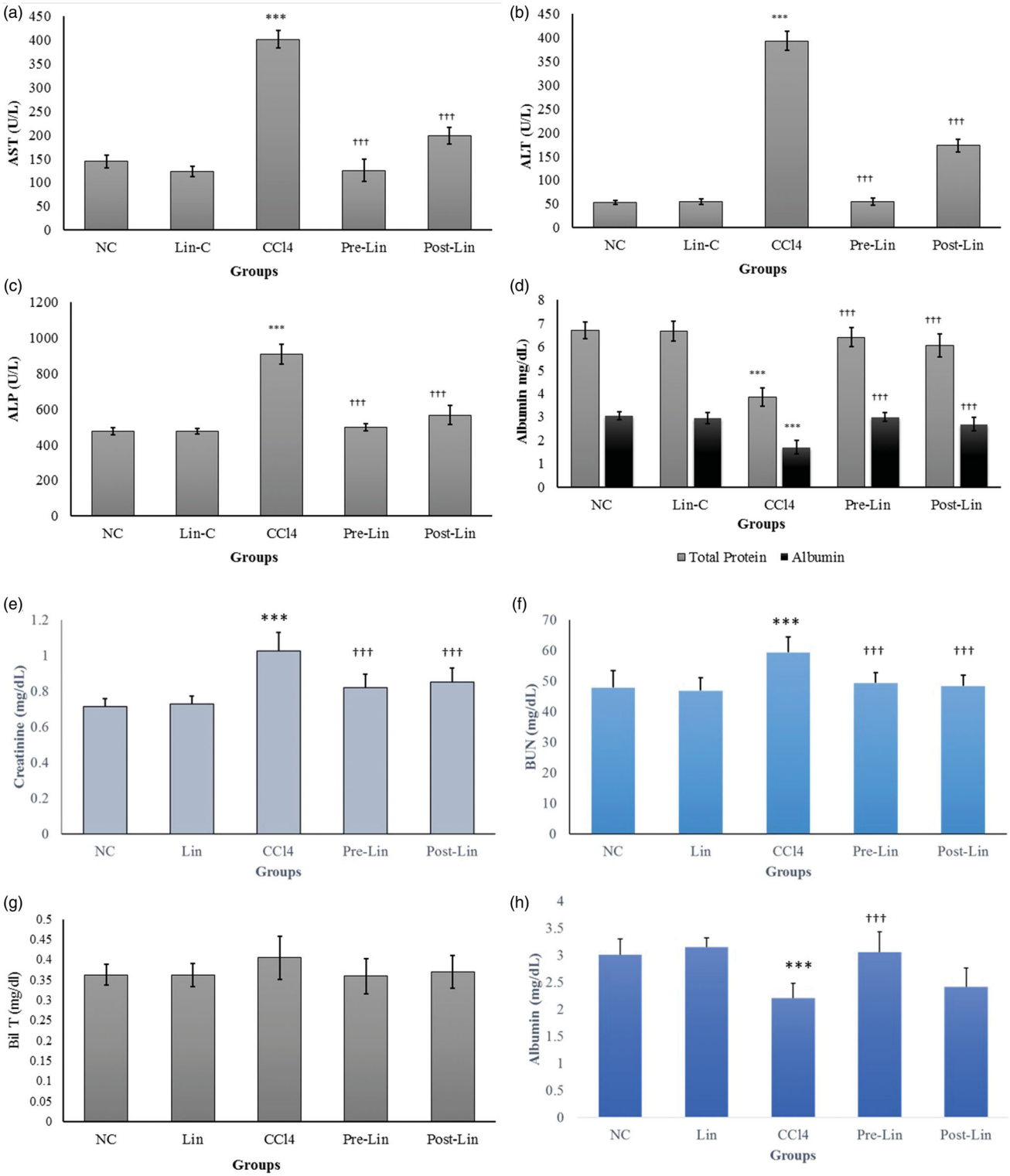

Figure 4. Protective and therapeutic effects of Lin on CCl_4 induce changes in the liver and kidney function with evaluation of (a) AST, (b) ALT , (c) ALP, (d) liver albumin, (e) Creatinine, (f) BUN, (g) total bilirubin, and (h) kidney albumin using commercially available kits. The results are expressed as mean ± standard deviation (SD) for the rats in each group. *** Shows the significance of the differences relative to the normal control group ($p < 0.001$). ††† Shows the significance of the differences relative to CCl_4 group ($p < 0.001$).

Protective and therapeutic effects of LIN on CCl4 -induced TNF-α and IL-6 productions

We investigated whether LIN decreases the production of the pro-inflammatory cytokines: TNF-α and IL-6 induced by CCl_4. The toxic effect of CCl_4 has resulted in significant ($p < 0.05$) higher IL-6, and TNF-α concentrations in liver and kidney tissues as compared to the control group. However, pretreatment with 25 mg/kg LIN caused significantly ($p < 0.05$) lower concentrations of these inflammatory in these liver and kidney tissues than those not treated (Figure 5(a,b)).

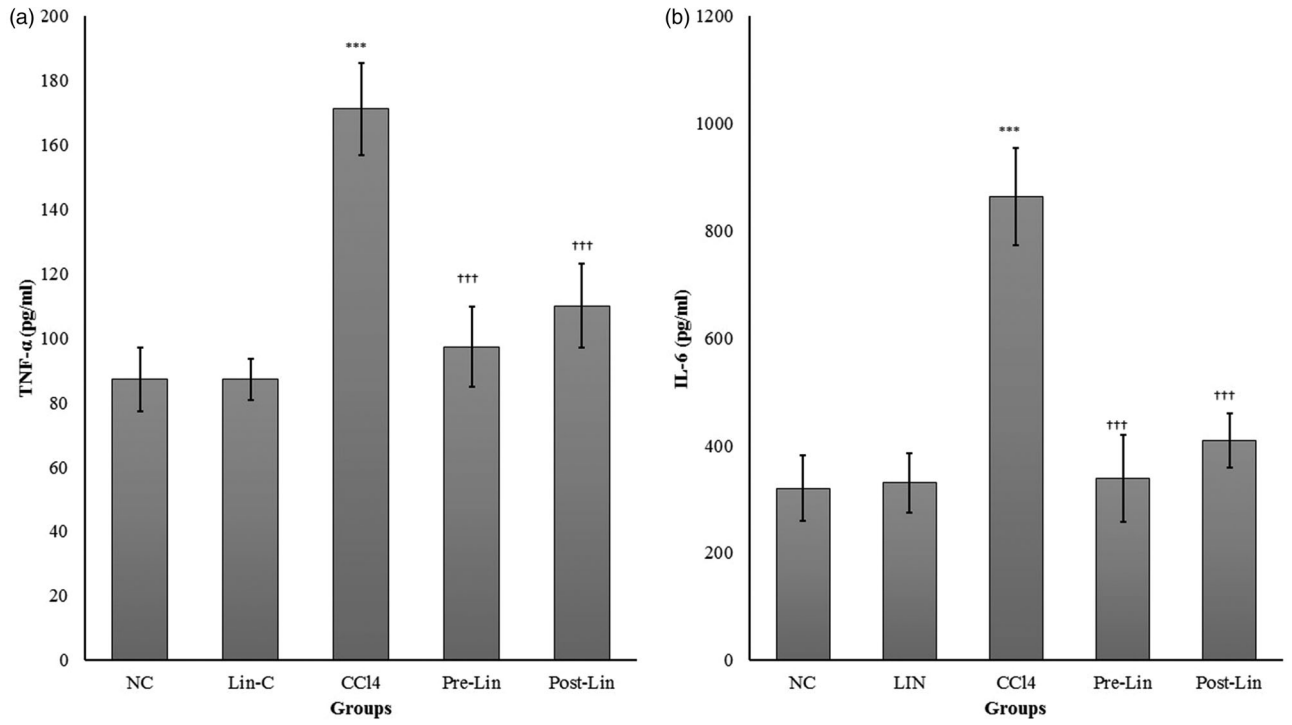

Figure 5. Protective and therapeutic effects of Lin on CCl_4 induce changes in the production of TNF-α and IL-6 in liver tissue of Wistar rats using commercially available kits. The results are expressed as mean ± standard deviation (SD) for the rats in each group. *** Shows the significance of the differences relative to the normal control group ($p < 0.001$). ††† Shows the significance of the differences relative to CCl_4 group ($p < 0.001$).

Table 1. Protective and therapeutic effect of linalool (Lin) on CCL_4 induced changes in hepatic and renal MDA, TAC, and antioxidant enzymes (SOD, GP_X, and CAT) activity.

Experimental groups	NC	Lin	CCL_4	Pre-Lin	Post-Lin
Liver					
MDA (nmol/mg protein)	0.10 ± 0.014	0.11 ± 0.012	0.34 ± 0.027***	0.10 ± 0.013 †††	0.13 ± 0.012†††
TAC (mmol/liter)	0.41 ± 0.05	0.41 ± 0.05	0.22±.04***	0.39 ± 0.05†††	0.34 ± 0.04†††
SOD (U/mg protein)	11.05 ± 0.54	11.07 ± 0.54	7.8 5 ± 0.15***	8.55 ± 0.17††	7.92 ± 0.49
GPx (U/mg protein)	4.69 ± 0.44	4.78 ± 0.40	3.39 ± 0.49***	4.58 ± 0.40†††	3.83 ± 0.40
CAT (U/mg protein)	86.17 ± 10.71	89.15 ± 10.66	41.84 ± 14.00***	66.88 ± 10.81††	58.87 ± 15.66†
Kidney					
MDA (nmol/mg protein)	2.2 ± 0.122	1.7 ± 0.20	24 2.2 ± 2.1***	13.06 ± 1.11†††	9.9 ± 1.8†††
TAC (mmol/liter)	414.8 ± 43	409.03 ± 45	263.8 ± 39***	356.2 ± 47†††	371. 7 ± 41†††

The results presented in the table are expressed as mean ± standard deviation (SD) for the rats in each group.
*** Shows the significance of the differences relative to the normal control group ($p < 0.001$).
†, ††, and ††† Show the significance of the differences to CCl_4 group ($p < 0.05$, 0.01, and 0.001, respectively).

Protective and therapeutic effect of LIN on CCl_4 -induced changes in hepatic nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) expression

Administration of CCl_4 with single dose increased mRNA expression of NF-κB (Figure 6). LIN alone decreased the expression of NF-κB. Co-administration of LIN and CCl_4 normalized such increase in NF-κB expression reported in CCl_4 group. These results indicate that co-administration of LIN with CCl_4 decreases NF-κB.

Discussion

Acute and chronic exposure to some organic solvents is known to cause hepatotoxicity and nephrotoxicity (Malaguarnera *et al.* 2012). In the past of several decades, oxidative stress has been found to be the man mechanism of action of a number of some solvent (Kum *et al.* 2007). Solvents are highly lipophilic, which rapidly absorb and distribute in the body (Fiserova-Bergerova 1985). The presence of the enzymatic systems necessary for solvent metabolism and the high levels of transition metals found in the liver and kidney have led to the oxidative stress in toxicity these agents (Brautbar and Williams II 2002, Al-Ghamdi *et al.* 2003). A number of enzymes might be responsible for an association between and oxidative stress and solvent exposure (Costa *et al.* 2006). One of the main enzyme systems in activation of solvent to reactive metabolite is cytochrome P450 enzymes which use molecular oxygen to oxidize some organic solvents (Furge and Guengerich 2006). This oxidation of solvents produces reactive oxygen species, especially very reactive intermediate of hydroxyl radical. In turn, hydroxyl radicals react with many cellular components such as proteins, polyunsaturated fatty acids, nucleic acids. Concentrations of cytochrome P450 are high in hepatic and renal tissue, and can be induced by xenobiotics, including

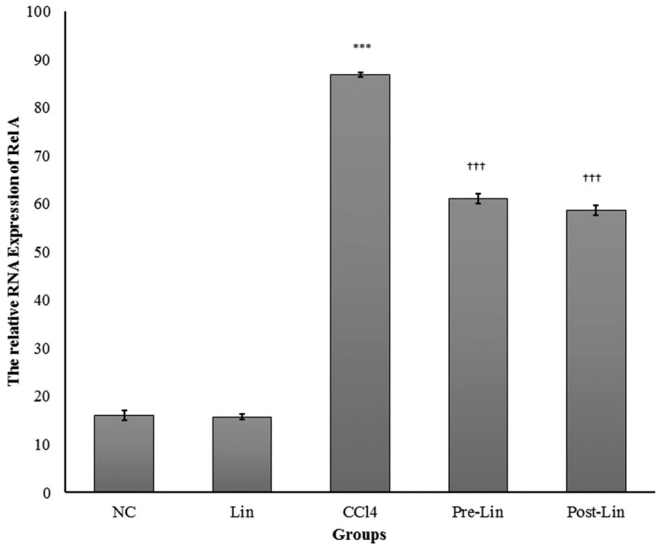

Figure 6. Protective and therapeutic effects of Lin on CCl_4 induce changes in the expression of NF-kB in liver tissue of Wistar rats using semi-quantitative RT-PCR analysis. RNA was extracted and reverse transcribed and RT-PCR analysis was carried out NF-kB expression as described in materials and methods. The results are expressed as mean ± standard deviation (SD) for the rats in each group. *** Shows the significance of the differences relative to the normal control group ($p < 0.001$). ††† Shows the significance of the differences relative to CCl_4 group ($p < 0.001$).

organic solvents (Pelkonen *et al.* 2008). Therefore, these enzymes play an important role in transforming organic solvents that have exposed with the liver and kidney. Finally, this can lead to oxidative stress with the production of free radicals and lipid peroxidation in the hepatic and renal tissues. CCl_4 as an organic solvent and agent in induction of liver and kidney damage mode, is activated by cytochrome P450, to form the trichloromethyl radical, CCl_3*. Trichloromethyl radical can bind to nucleic acids, proteins, and lipids. Our result in the current study showed that exposure with CCl_4 led to oxidative stress and lipid peroxidation in hepatic and renal tissues. These data are consistent with previously published data examining effect of CCl_4 on liver and kidneys (Muriel 1998, Tirkey *et al.* 2005). Due to the importance of the cytochrome P450 enzymes in activation of xenobiotics, assessment of the potential of compounds which reversibly inhibit cytochrome P450 enzymes can be an important strategy in reduction of toxicity. Previous studies have been suggested that linalool is weak competitive inhibition of cytochrome P450 enzymes in rat liver microsomes (Ganzera *et al.* 2006, Noskova *et al.* 2016), therefore, this compound can reduce the toxicity of substances that exert their toxicity from this pathway. In our study, pretreatment and post treatment with linalool showed a significant decrease in CCl_4-induced oxidative paraments. Probably inhibition of the cytochrome P450 by linalool could be effective in creating this effect.

There is powerful evidence that reactive species (RS) is involved in oxidative stress as a main mechanism by which industrial solvents induce liver and kidney damages (Jyothi *et al.* 2012). Due to RS high reactivity, these intermediates prone to cause damage to any type of molecule within the cell (Bergamini *et al.* 2004). Due to oxidative damages to the cellular components, the antioxidant defense in the human body becomes overwhelmed and lead to toxicity responses such adaptive, inflammatory, reparative and injurious processes (Kehrer and Klotz 2015). Oxidative stress has been considered to be closely associated with nuclear factor (NF)-κB (Niederberger and Geisslinger 2008). A vicious cycle has been reported between the oxidative stress and NF-κB pathway. For the activation of the NF-κB signaling pathway, oxidative stress is crucial, it results in the aggravation of oxidative stress (Morgan and Liu 2011).Our results at gen, molecular cellular and tissue levels showed that exposure with industrial solvent CCL_4 caused all toxicity responses mentioned in above and created a state of oxidative stress condition in these two organs. Today, antioxidants as potential medical countermeasures are used for toxic industrial chemicals (McElroy and Day 2016). Many of these agents produce antioxidant properties indirectly or even paradoxically (Devasagayam *et al.* 2004). Direct acting antioxidants that scavenge reactive species may often generate a reactive product (Winterbourn 2008). This condition happens with SOD that dismutate superoxide and produces hydrogen peroxide (Ighodaro and Akinloye 2018). Previous studies have demonstrated the high antioxidant potential of linalool on reactive species scavenging in comparison with ascorbic acid (Seol *et al.* 2016). These studies confirmed that the linalool can be used in the synthesis of several type of compound with ability to act as antioxidant (Jabir *et al.* 2018). They suggested that linalool as a medicine drug can be used in the treating several types disease related to oxidative stress (Seol *et al.* 2016). Our results confirmed that the linalool has protective and therapeutic effect against CCl_4-induced hepatic and liver damages.

Exposure to drugs, chemicals, mechanical, and thermal injuries cause inflammation as a tightly regulated immune-protective response to combat these agents (Kidd and Urban 2001). Our results showed that CCL4 induces the production of pro-inflammatory cytokines such as IL-6, and TNF-α from liver and kidney tissues which these data are consistent with previously published data in this regard (Dai *et al.* 2018b).

Inhibition of inflammation is always one of the most important actions in controlling the toxicity effect of these factors. Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to suppress prostaglandin E2 (PGE2) production through the inhibition of cyclooxygenase-2 (COX-2). There is a strong evidence that have shown, the prolong exposure to this drug class has been associated with serious and sometimes life-threatening side effects (Mbonye *et al.* 2008). Hence it is necessary to find out alternative anti-inflammatory agents with comparative or more efficacy than NSAIDs but with fewer side effects. All kinds of inflammatory conditions have been treated with the natural products. These natural products later become the counter stones for producing that main ani-inflammatory drug such as Aspirin. Therefore, the exploration in the natural products to find out the bioactive compounds can be quite promising and resulting in the discovery of numinous secondary metabolites with extra ordinary bioactivities (Attiq *et al.* 2018). Our results showed that linalool can inhibit the expression and production against CCl_4-induced induction of inflammatory mediators.

Conclusion

In conclusion, the present study was designed to yield quantitative evidence of the protective and therapeutic properties of linalool against CCl_4-induced hepatic and renal damages. The positive effects of linalool such reduction of oxidative stress and expression of anti-inflammatory mediators and enhancement of antioxidant defense were proved in this study. However, further studies are needed to clarify the mechanism of the protective and therapeutic effect of linalool against liver and kidney damages. To our knowledge, this is the first evidence that linalool protects and ameliorates against CCl_4-induced acute hepatotoxicity and nephrotoxicity.

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